REMARKS

Claim Objections

The Office Action dated November 18, 2008, objects to claim 1 stating that the full term for alpha-MEM is recited in parentheses after first presenting the term as an abbreviation. Applicants note that this correction was already made in the amendment submitted on August 15, 2008. Withdrawal of the objection is respectfully requested.

Claims 6-8 have been added. These claims are supported by the specification at, for example, paragraphs 0023 and 0027 as well as Table 1.

Rejections under 35 U.S.C. §103

The Office Action rejects claims 1-5 under 35 U.S.C. §103(a) as obvious over Erices *et al.* (British Journal of Haematology 2000, 109: 235-242) in view of Nishikawa *et al.* (publ. no. US 2004/0235160). Applicants respectfully traverse.

The present invention is directed to a method of isolation of mesenchymal stem cells (MSCs) from *cryopreserved* umbilical cord blood (UCB). None of the references, whether taken alone or combined, disclose or teach the claimed method of isolating and culturing MSCs from cryopreserved UCB. Thus, the Office Action fails to establish *prima facie* obviousness because the references do not teach each and every limitation of the claims. The examiner's assertion that cryopreservation is a well known method ("...at the time of the claimed invention, cryopreserved cord blood was well known and used source of cord blood", last paragraph, page 3 of Office Action) does not remedy this situation. Cryopreservation is not always suitable, particularly for a source of live, culturable cells. For example, it is known in the art that cryopreservation can render sensitive patient (or primary) tissue samples and the resulting primary cells unculturable due to the damage sustained upon the freeze-thaw cycle. Accordingly, one would not necessarily expect cryopreserved UCB to be a source of MSCs. For this reason as well,

prima facie obviousness is not established and the rejection should be withdrawn.

Further, as explained below, the combination asserted by the Office Action does not render the claims obvious because a person skilled in the art would not combine the references as set forth and, even if combined, there would be no reasonable expectation of success. Accordingly, the rejections should be withdrawn.

The Office Action states that Erices *et al.* teaches harvesting and preparing mesenchymal progenitor cells (MPCs) from UCB. Mesenchymal *progenitor* cells, however, give rise to cells of a *mesenchymal-like* phenotype (Abstract and page 24, Erices *et al.*)—namely "mesenchymal-like cells [MLC]" (page 240, 1st column, Erices *et al.*) or "fibroblastoid (MLC) cells" (page 241, 1st column, Erices *et al.*)—as opposed to *actual* MSCs of the present invention. MSCs are defined in the present specification as follows: "...in the case of stem cells isolated and cultured in accordance with the present invention, CD34, CD45 and CD14, which are characteristic indicators of hematopoietic stem cells showed negative reactions, [while] SH2, SH3, CD29 and CD44, which are characteristic indicator[s] of mesenchymal stem cells, showed positive reactions..." (paragraph 0026 and table 1, present application). Erices *et al.* does not indicate that isolated cells have the CD44 marker (page 241, 1st column, Erices *et al.*), and not having this marker is indicative of a *haemotopoietic* phenotype (emphasis added, Abstract, Wexler *et al.*, British Journal of Haematology 2003, 121: 368-374).

Further, only 25% of cord blood harvests of Erices *et al.* gave rise to an adherent layer that differentiated into fibroblastoid MLC cells (page 241, 1st column, Erices *et al.*). MSC-like cells that were never cryopreserved and were isolated with difficulty from UCB is not the same as *reliably* isolating *actual* MSCs from cryopreserved UCB as in the present invention. Additionally, as shown in Erices *et al.*, MSCs were isolated from pre-term UCB. In contrast, the present invention is useful in isolating MSCs from full-term cryopreserved UCB. Applicants' claimed method successfully isolates MSCs from full-term cryopreserved UCB reliably at a rate of 98% compared to 2% in the field (Table 2 of the present application). Thus, the differences in methods between the present invention, Erices *et al.*, and the other prior methods is significant in allowing for the isolation of MSCs from cryopreserved UCB.

The Office Action states that one skilled in the art would recognize that CD133 is a known marker of MSCs and that the culture media utilized is commonly subject to optimization. The cells isolated in the present invention contain the CD133 marker, which proves the cells isolated to be *true* MSCs. In contrast, the Erices *et al.* group is silent with respect to the CD133 marker. Since Erices *et al.* admit to only isolating *mesenchymal-like* cells, it cannot be assumed that this marker is present.

As for the culture media, Erices *et al.* isolate MPCs from UCB via a method that is conventionally used for isolation of bone marrow cells (see Majumdar *et al.*, Journal of Cell Physiology 1998, 176(1): 57-66). Erices *et al.* does not disclose or use a medium that includes Stem Cell Factor, GM-CSF (granulocyte-macrophage colony-stimulating factor), G-CSF (granulocyte colony-stimulating factor), IL-3 (interleukin-3), and IL-6 (interleukin-6) as required by claim 1. Nishikawa *et al.*, however, does not cure deficiencies of the Erices *et al.* approach. The Office Action states that Nishikawa *et al.* is cited only to show the presence of additional components in the culture media. However, a disclosure that some culture media contain additional components does not, by itself, make the reference combinable with Erices *et al.* in a manner that renders the claims obvious. A proper analysis of Nishikawa *et al.* reveals that one skilled in the art would not be motivated to combine Nishikawa *et al.* (which relates to isolating HSCs *not* MSCs as the Office Action indicates) with Erices *et al.* (which is related to MSCs) as outlined in the Office Action.

Further, even if one combined these references, there would be no reasonable likelihood of success. Refer to MPEP 2143.01 section III (emphasis added): "The mere fact that references *can* be combined or modified does *not* render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art". Also refer to MPEP 2143.01 section V: "If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification".

A person of ordinary skill in the art would recognize that different additives and cytokines are utilized to grow various types of cells and to differentiate cells into specific cell types. Nishikawa *et al.* utilizes a mixture containing a particular stimulating factor,

gp130, to specifically derive the hematopoietic stem cell (HSC) population from a culture. Not only does the present invention not utilize this stimulating factor, but using gp130 would limit the cells isolated to be exclusively HSCs, which are "cell[s] having the ability to differentiate into all lineages of the *blood cells*" (emphasis added, paragraph 0021, Nishikawa *et al.*) and are a less diverse cell type than the pluripotent MSCs. A person seeking to isolate cryopreserved MSCs would, therefore, not look to Nishikawa *et al.* in seeking to modify or improve the Erices *et al.* method.

Further, Nishikawa *et al.* "utilize stromal cells [as an additive] which provide a suitable environment for the maintenance and expansion of hematopoietic cells to support the maintenance and expansion of hematopoietic stem cells without differentiation" (paragraph 00006, Nishikawa *et al.*). Thus, the supplements utilized by Nishikawa *et al.* are unlike those of the present invention, which do not utilize these feeder systems. Further, the MSCs used in Example 5 of Nishikawa *et al.* are "harvested and cultured from normal human bone marrow" and not cryopreserved UCB as in the present invention. Additionally, these supplements are used for culturing HSCs *not* MSCs as in the present invention. Thus, using gp130 (which is essential to the teaching of Nishikawa *et al.*) in the present invention is not possible because that would lead to stimulating alternate differentiation pathways resulting in the isolation of a more limited cell type, the HSCs (which only differentiate in blood cells), rather than the MSCs, which are "primitive cells that are able to differentiate into bone, cartilage, adipose tissue, nerve, and muscle" (lines 15-16, present application). Thus, if one combined the teaching of Nishikawa *et al.* with Erices *et al.*, the modification would be unsatisfactory for the isolation of cryopreserved MSCs and, in fact, would not work for its intended purpose.

Accordingly, the rejections should be withdrawn because a person skilled in the art would not combine the references as set forth and, even if combined, there would be no reasonable expectation of success.

Lastly, the Office Action has not adequately or correctly consider the state of the art that describes the need for methods to isolate cryopreserved MSCs from UCB. The state of the art teaches that one would *not* expect to isolate MSCs from UCB and, at the very least, severely limits the scope of the Erices *et al.* method. The prior art does not

discuss methods for isolating MSCs from cryopreserved UCB. In fact, other research groups cast doubt on the results of the Erices *et al.* group, which used fresh samples (i.e., samples that were never cryopreserved). For example:

- Mareschi *et al.* (Haematologica 2001, 86: 1099-1100) state that "our results suggest that early fetal blood is rich in MSCs but [full] term UCB is not" (Abstract, Mareschi *et al.*) and "Erices *et al.* (2000) showed that MSCs could be separated from UCB but this was mainly from *preterm* UCB" (emphasis added, page 124, right column, Mareschi *et al.*). This casts significant doubt on the rate of success Erices *et al.* achieve with their methods for isolating MSCs from UCB. Further, Mareschi *et al.* notes that isolating MSCs from UCB has not been accomplished by the Erices *et al.* group since UCB is a source of HSCs only: "it was possible to isolate MSCs from bone marrow but not from UCB" and "BM [bone marrow] contained mesenchymal stem cells that could easily be expanded and induced to differentiate for therapeutic use while the UCB adherent monolayer displayed the morphology and the characteristics of hematopoietic cells and not those of mesenchymal cells" (page 1099, Mareschi *et al.*).
- Romanov *et al.* (Stem Cells 2003, 21: 105-110) indicates that Erices *et al.* may not have isolated mesenchymal cells via the method disclosed since "umbilical cord blood is a rich source of hematopoietic stem/progenitor cells and does not contain mesenchymal progenitors" (Abstract).

The Office Action incorrectly indicates the importance of the invention as obtaining blood from the umbilical cord (UC). Instead, the invention is in isolating MSCs from cryopreserved UC *blood* that is unique as scientific literature plainly states that "adult BM [bone marrow] is a reliable source of functional cultured MSC, but CB [umbilical cord blood] and PBSC [peripheral blood stem cell collections] are not" (page 368, Wexler *et al.*). Others have also been unable to isolate MSCs from UCB. For instance, Romanov *et al.* isolated MSCs from a different source than the present invention since they "attempt[ed] to isolate MSCs from the *subendothelial layer* of umbilical cord vein" (emphasis added, Abstract, Romanov *et al.*) and did not isolate MSCs from UC *blood* as in the present invention.

CONCLUSIONS

Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. Accordingly, Applicants request that the Examiner issue a Notice of Allowance for pending claims 1-8 and that the application be passed to issue. Applicants respectfully request that a Notice of Allowance of pending claims 1-8 be timely issued in this case.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is hereby invited to telephone the undersigned at the number provided. The Commissioner is authorized to charge any deficiency in any patent application processing fees pursuant to 37 CFR §1.17, including extension of time fees pursuant to 37 CFR §1.17(a)-(d), associated with this communication and to credit any excess payment to Deposit Account No. 22-0261.

Respectfully submitted,

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